REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. CLAIM STATUS AND AMENDMENTS

Claims 1-7, 9-11, 13-17 and 19 were pending in this application when last examined.

Claims 1-7, 9 and 10 were examined on the merits and stand rejected.

Claims 11, 13-17 and 19 were withdrawn as non-elected subject matter. Applicants reserve the right to file a Continuation or Divisional Application on any withdrawn subject matter.

Claims 6 and 8-10 are cancelled without prejudice or disclaimer thereto.

Claim 1 is amended. Support for the amendment to the preamble of "constructing a DNA vector having a cDNA synthesized from an mRNA" can be found in Examples 3, 4 and 8 on pages 23, 24 and 27 of the specification. In particular, it is noted that the claimed method is capable of synthesizing and cloning cDNA with either "dG" or "(dT)ndG". Thus, the former limitation in claim 1 of "5'-end nucleotide of (dT)ndG" is unnecessary. Rather, the claimed invention is directed towards synthesizing and cloning cDNA in a DNA vector of circular form by simple procedures without additional linkers sequences.

Support for the limitation in step (i) can be found in claim 9 and page 15, line 16, to page 16, line 2, of the specification. Support for the amendment to step (iii) can be found in claim 6. Support for the amendments to step (iv) can be found in Example 1 part (5) on page 19 of the specification and in claim 10.

No new matter has been added.

II. INFORMATION DISCLOSURE STATEMENT

The Examiner is respectfully requested to return an initialed copy of the PTO-1449 form filed May 5, 2008.

III. INDEFINITENESS REJECTION

On pages 2-3 of the Office Action, claims 1-7, 9 and 10 were rejected under 35 U.S.C. § 112, second paragraph, as indefinite. This rejection is overcome, as applied to the remaining amended claims for reasons which are self-evident.

IV. OBVIOUSNESS REJECTION

On pages 3-7 of the Office Action, claims 1-7, 9 and 10 were rejected under 35 U.S.C. § 103(a) as obvious over Chenchik et al. in view of Mueller et al. Applicants respectfully traverse this rejection.

In the method of Chenchik et al. ("template-switching method"), template switching oligonuleotide is added at the 3'-end of the first-strand cDNA. Then, the 3'-end of the template switching oligonuleotide is ligated with the 5'-end of a double stranded DNA vector with T4 DNA ligase to form a circular vector.

On the other hand, in the claimed invention, the mRNA/cDNA heteroduplex is circularized by using T4 RNA ligase. In this regard, the Examiner alleges that T4 RNA ligase is described in Chenchik et al. (Office Action, page 6, rejection to claim 6). However, this is incorrect. Chenchik et al. (col. 14, line 62 to col. 15, line 1) refers to "T4 DNA ligase".

For ligation with T4 DNA ligase, artificial sequence (template switching oligonuleotide) is necessary for a correct ligation, as in the method of Chenchik et al. Mueller et al. also artificially added $(dA)_n$ at the 3'-end of the first strand cDNA. The present inventors, on the other hand, have found that T4 RNA ligase makes it possible to correctly ligate the rnRNA/cDNA heteroduplex and the double-stranded DNA for circularizing without any additional sequence (see Fig. 1, for example).

Applicants further note that the cDNA synthesized by the present invention has an additional dG or $(dT)_ndG$ at the 5'-end (please see Examples 3, 4 and 8). This means that the 3'-end of the first strand cDNA has additional dC. However, this additional nucleotide is not artificial. The first strand cDNA synthesized from mRNA with CAP structures naturally have dC at the 3'-end. This is another feature of this invention.

Further, Applicants note that the novelty of the present invention is reinforced by the presence of a number of publications in the art. A copy of the Abstracts from these publications are attached herewith (Attachments A, B and C).

Thus, Applicants note that none of the cited references teach or suggest a method for synthesizing cDNA using T4 RNA ligase and without the necessity of adding additional artificial sequence.

For the above-noted reasons, this rejection, as applied to the remaining amended claims, is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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November 19, 2008

ATTACHMENTS

A. Ohtake, H. et al., "Determination of the Capped Site Sequence of mRNA based on the Detection of Cap-Dependent Nucleotide Addition Using an Anchor Ligation Method", DNA Research, 2004, 11(4): 305-309. (Abstract)

B. Kato, S. et al., "Vector-Capping: A Simple Method for Preparing High-Quality Full-Length cDNA Library", DNA Research, 2005, 12(1): 53-62. (Abstract)

C. Oshikawa, M. et al., "Fine Expression Profiling of Full-length Transcripts using a Size-unbiased cDNA Library Prepared with the Vector-capping Method", DNA Research, 2008, 15(3): 123-136. (Abstract)

DNA Research

- Oxford Journals
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- DNA Research
- Volume 11, Number 4
- Pp. 305-309

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DNA Research 2004 11(4):305-309; doi:10.1093/dnares/11.4.305

Determination of the Capped Site Sequence of mRNA Based on the Detection of Cap-Dependent Nucleotide Addition Using an Anchor Ligation Method

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The sequence analysis of the 5' ends of cDNAs prepared using the anchor ligation method has revealed that most of the full-length cDNAs have an additional dGMP at their 5' end that is absent in the corresponding genome sequence. Using model RNA transcripts with cap analogues possessing 7-methylguanosine and adenosine, the base of the added nucleotide has been shown to be complementary to the base of the cap analogue, suggesting that the cDNAs possessing an additional dGMP are derived from intact mRNAs with the cap structure. On the other hand, cap-free RNA did not produce cDNA with an extra dGMP. These findings suggest that we can determine whether or not the cDNA starts from the capped site

Attachment A

sequence of mRNA based on the presence or absence of an additional dGMP at the 5' end of the cDNA synthesized using the anchor ligation method. This approach will be useful to determine the capped site sequence of mRNA, thus, to identify transcription start sites.

Key words: full-length cDNA; cap; transcription start site; anchor ligation

This article has been cited by other articles:

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- DNA Research
- Volume 12, Number 1
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DNA Research 2005 12(1):53-62; doi:10.1093/dnares/12.1.53

Kazusa DNA Research Institute

Vector-Capping: A Simple Method for Preparing a High-Quality Full-Length cDNA Library

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Full-length cDNAs play an essential role in identifying genes and determining their promoter regions. Here we describe a simple method for constructing a full-length cDNA library, which has the following advantages: (i) it consists of only three steps including direct ligation between a vector and a cDNA strand using T4 RNA ligase, (ii) it contains neither a PCR process generating mutations nor restriction enzyme treatment causing truncation of cDNA, (iii) the intactness of cDNA is assured due to the presence of an additional dGMP at its 5' end, (iv) approximately 95% of cDNA clones are full-length when cultured cells or fresh tissues are used, (v) several micrograms of total RNA without mRNA purification is sufficient for preparation of a library containing $>10^5$ independent clones, and (vi) a long-sized full-length cDNA up to 9.5 kbp can be cloned. This method will accelerate comprehensive gene analysis in a variety of eukaryotes.

Attachment B

Key words: full-length cDNA; cDNA synthesis; transcription start site; T4 RNA ligase

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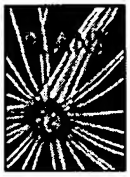

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Comparasite: a database for comparative study of transcriptomes of parasites defined by full-length cDNAs
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A large-scale full-length cDNA analysis to explore the budding yeast transcriptome

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[Full Text] [PDF]

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Fine Expression Profiling of Full-length Transcripts using a Size-unbiased cDNA Library Prepared with the Vector-capping Method

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Received 24 December 2007 ; accepted 10 April 2008.

Recently, we have developed a vector-capping method for constructing a full-length cDNA library. In the present study, we performed in-depth analysis of the vector-capped cDNA library prepared from a single type of cell. As a result of single-pass sequencing analysis of 24 000 clones randomly isolated from the unamplified

library, we identified 19 951 full-length cDNA clones whose intactness was confirmed by the presence of an additional G at their 5' end. The full-length cDNA content was >95%. Mapping these sequences to the human genome, we identified 4513 transcriptional units that include 36 antisense transcripts against known genes. Comparison of the frequencies of abundant clones showed that the expression profiles of different libraries, including the distribution of transcriptional start sites (TSSs), were reproducible. The analysis of long-sized cDNAs showed that this library contained many cDNAs with a long-sized insert up to 11 199 bp of golgin B, including multiple slicing variants for filamin A and filamin B. These results suggest that the size-unbiased full-length cDNA library constructed using the vector-capping method will be an ideal resource for fine expression profiling of transcriptional variants with alternative TSSs and alternative splicing.

Key words: full-length cDNA; expression profile; transcriptional start site; alternative splicing; antisense transcript

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